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Precision and Bias of Various Soybean Dry Matter Sampling Techniques[1]

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ABSTRACT

A balance between sample sizes sufficient to detect real differences and resources necessary to obtain and process samples is a continual problem for scientists. In the first year, this study assessed the precision and bias associated with three subplot sampling techniques used for estimating soybean [*Glycine max* (L.) Merr.] shoot dry matter production: (a) random 1 m of row, (b) random 0.3 m of row, and (c) four randomly selected plants. The experimental design was a split-split plot. Three cultivars (Braxton, Coker 338, and Davis) were the whole plots, and three sampling date plots were the subplots. Measurement of the total plant dry matter within the subplots as well as the dry weight obtained by three sampling methods constituted the four split-split plots. When dry matter data were averaged over time and cultivar, the coefficients of variation (CV) for the sampling techniques (a), (b), and (c) were 0.187, 0.417, and 0.386, respectively. The CV was 0.088 when the 20-m^2 subplot (27 m of row) was measured. The 1-m technique did not significantly overestimate dry matter production relative to the entire subplot measure, but the 0.3-m and four-plant (4-P) techniques significantly overestimated dry matter production. Cultivar, date of sampling, and cultivar × date-of-sampling interaction effects in the analysis of variance were significant at $P \leq 0.05$ only when data from the entire subplot or 1-m sampling technique were used. A second study in 1985 also showed that the 0.3-m and 4-P sample techniques were poor for both precision and accuracy. This second study also compared the precision and bias of 1- and 2-m samplings as well as the precision and bias associated with different technicians. The 1- and 2-m techniques gave equally unbiased results, but the 1-m technique did not give equally good precision (CV). Comparison of techniques was not significantly affected by technicians. We concluded that adequate accuracy and precision were obtained by use of the 1-m or 2-m sampling technique, but not by the 0.3-m or 4-P techniques.

Additional index words: **Soybean dry matter, *Glycine max* (L.) Merr., Coefficient of variation, Sign test, Plant sample size.**

Soybean [*Glycine max* (L.) Merr.] has worldwide importance, and investigators are interested in many aspects of its growth and culture. Studies on nutrient status (10), yield (2,4,8), nitrogen fixation and annual balance (3,9,11,12,13,14), and residue or dry matter production (5,7) have been of particular interest. Accurate determination of soybean dry matter is important for all of these interests. Yield data for soybean are typically taken from > 10 m of row per experimental unit, and coefficients of variation (CV's) are commonly < 0.10 (8,10,12). However, shoot samples for dry matter and N accumulation estimates are frequently taken from 1 m of row or less (5,7,13), and CV's of these estimates are often 0.20 or greater (5). Additionally, there is bias that might be associated with various sampling techniques.

Although subsample size and technique for obtaining soybean shoot samples are numerous, they usually involve either the random selection of a number of plants and the multiplication of the per plant value by a population estimate, or the sampling of a section of row and multiplication by a factor for conversion to a unit-area basis (5). Statistical aspects of subsampling are discussed under the topic of two-stage sampling by Steel and Torrie (15).

Carter et al. (5) evaluated the precision of various sampling techniques, but their experiments were in different locations and different years. Thus, sampling techniques could not be directly compared. In a later study, Carter et al. (6) reported the optimum sampling size of soybean to be 2 m of bordered row. However, we found no investigations in which the within-plot sampling estimates were actually compared to the subsequent entire subplot measurements to allow for an estimate of bias. Therefore, we investigated the precision and bias of four sampling techniques.

MATERIALS AND METHODS

A 2-yr field study was conducted on Norfolk loamy sand (fine-loamy, silicious, thermic Typic Paleudult). In 1984, a split-split plot design with four replicates of a randomized complete block design was used (Fig. 1). Soybean cultivars (Davis, Braxton, and Coker 338, maturity groups VI, VII, and VIII, respectively) were arranged as whole plot treatments. The split-plot treatments (sampling dates) were 20-m^2 subplots that were harvested on three separate dates. The split-split plots were three sampling techniques and a complete measure of the dry matter in a subplot.

Fertilization consisted of 15, 84, and 1121 kg ha^{-1} of P, K, and dolomitic lime, respectively. 'Treflan' (trifluralin—α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toludine) and 'Lexone' [metribuzin—4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] were applied prior to planting at the rate of 0.7 and 0.4 L ha^{-1}, respectively. Soybean cultivars were planted on 18 May at 33 seeds m^{-2}, and each subplot contained eight rows 4.6 m in length on 0.75-m spacing. Overhead irrigation was used as needed to prevent drought stress. Samples were taken during the vegetative through podfill stages of growth at 89, 115, and 138 days after planting (DAP). On each sampling date, 3 sampling techniques were used within each subplot (20 m^2) before the entire 20-m^2 subplot was harvested (Fig. 1): (a) randomly selected 1 m of row, (b) randomly selected 0.3 m of row, and (c) four randomly selected plants. One sample was obtained for each of three sampling techniques for each subplot. Rows for subsampling were randomly selected from the middle six rows, and the meter stick was placed within the middle two-thirds of the row. The four randomly selected plants (4-P) were obtained by placing a meter stick into four locations of the subplot and harvesting the plant nearest to the 1-m mark. Plants for the 0.3-m technique were obtained by harvesting all plants between the 0.4- and 0.7-m marks. Plants for the 1-m technique were obtained by harvesting all plants along the length of the stick. Total harvest of the subplot was considered to be the fourth sampling technique. Plot ends were trimmed to obtain a 20 m^2 (27 m of row on 0.75-m spacing) subplot. Plant samples were dried at 70°C and measured for dry weight. Seed yield and fallen litter were measured on a fourth subplot on a fourth sampling.

In 1985, sampling techniques were again compared to the total plot dry weight results, but only one sampling date (97 DAP) and one cultivar (Coker 368) were used. Three technicians (whole plot) sampled six replicates, for a total of 18 whole plots of a randomized complete block design. Split-plot factors were again subsampling methods (including har-

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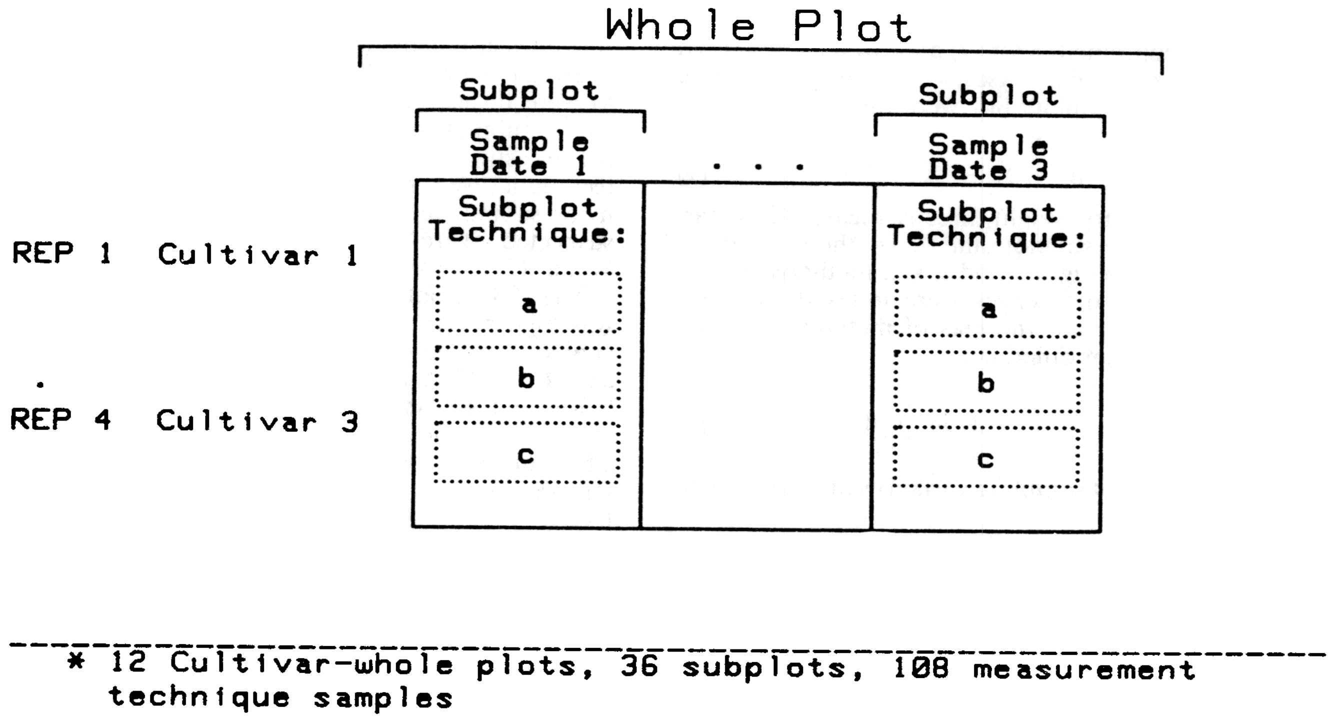

Fig. 1. A schematic of the experimental design and subplot sampling techniques.

vesting the entire plot). A 2-m subsample was taken in addition to the three techniques used in 1984. For both years, data were analyzed by analysis of variance (ANOVA) as outlined by Steel and Torrie (15). Sampling techniques were compared in pair-wise comparisons.

RESULTS AND DISCUSSION

One of the major problems associated with large experimental errors and poor precision is the inability to detect treatment differences. This problem is clearly shown in Table 1. The 0.3-m and 4-P techniques gave no $P < 0.05$ for replication, cultivar, date, or cultivar × date. Yet, subplot differences among date, cultivar, and cultivar × date means were > 31, 35, and 39% of the grand mean, respectively ($P < 0.01$). True differences of this magnitude are important to the interpretation of experimental results and proper soil management. Thus, it is of considerable experimental and resource-allocation importance that the 1-m sampling technique also produced *P*-values of < 0.01 for all sources of experimental variation except replications. From an experimental precision standpoint, the 1-m sample is clearly superior to the 0.3-m or 4-P techniques for these soybean, which had a mean yield of 2.01 mg ha^{-1}. The seed yield along with vegetative dry matter and litter were obtained on a fourth sampling date, but the data were not used in these analysis.

Measures of precision are presented in Table 2. The CV for the mean obtained from the sampling of the subplot for all cultivars and dates was 0.088; a value that is low relative to those reported by Carter et al. (5). This lower CV occurs because the subplot was large relative to typical dry matter sample size. Reduction of the sample size from 27 m of row to 1 m of row increased the CV by a factor of 2.1. However, reduction of the sampling size to 0.3 m of row or use of the 4-P technique increased the CV by more than fourfold. The root error mean square (REMS) for the 1-m technique only increased by 2.2-fold over the subplot, while either the 0.3-m or 4-P techniques increased the REMS by over sixfold.

There was no significant sampling method × cultivar or subsampling method × date interaction. Thus,

Table 1. Analyses of variance of soybean shoot dry weight as determined with data from various sampling techniques in 1984.

		Sampling techniques							
		SP†		1-m		0.3-m		4P	
Source	df	MS	*P*-value‡	MS	*P*-value	MS	*P*-value	MS	*P*-value
Replication		1.68	.0092	2.37	.2756	25.55	.2084	2.77	.8909
Cultivar		5.80	.0001	9.92	.0092	0.16	.9900	43.68	.0564
Date		6.31	.0001	14.44	.0018	7.79	.6143	5.68	.6603
Cultivar × date	4	1.93	.0027	7.81	.0073	12.94	.5214	5.90	.7791
Pooled§ error	24	0.35		1.73		15.66		13.44	

† SP = subplot (27 m of row), 1-m = 1-m row, 0.3-m = 0.3-m row, 4P = four plants, MS = mean squares.
‡ *P*-value = probability of a greater *F* value under the null hypothesis.
§ Analysis of the 1984 data showed that errors (a) and (b) of the standard split-plot ANOVA were not significantly different (*F* values were 1.0 or less), and a combined error (a) and (b) term was used.

Table 2. Means and estimates of error by four methods of soybean sampling in 1984.

Method	Mean	REMS†	CV	σ_s‡
Subplot		0.591	0.088	0
1-m row		1.314	0.187	1.391
0.3-m row		3.957	0.417	3.662
Four-plant		3.666	0.386	3.110

† REMS = root error mean square, CV = coefficient of variation.
‡ σ_s is derived form Eq. [3].

cultivar and dates were pooled for most of the analyses, and a combined error (a) and (b) term was used for all of the reported analyses. Variation of a sampling method within subplots (σ_s) were directly estimated from the 36 differences:

$$d_s = \hat{X}_s - X, \qquad [1]$$

where X = the dry weight directly measured by harvesting the entire subplot; and $\hat{X}_s$ = the estimate of X based on sampling method s, i.e., methods (a), (b), and (c). The 36 differences were indexed by i, $i = 1, \ldots, 36$ (i.e., d_{si}), and the bias of methods was estimated as the mean difference:

$$\text{bias} = \bar{d}_s = 1/36 \sum_{i=1}^{36} d_{si}. \qquad [2]$$

The within subplot sampling variances of methods can be estimated as

$$\hat{\sigma}_s^2 = \frac{\sum_{i=1}^{36} (d_{si} - \bar{d}_s)^2}{35}. \qquad [3]$$

When a subplot sampling technique is used, the error mean square (EMS) is $\sigma_s^2 + \sigma^2$, because within-plot (σ_s^2) and among-plot (σ^2) sources of variation are additive. Thus, σ_s^2 is the appropriate measure of the error associated with sampling techniques as opposed to the error associated with field and cultivar variations. Since there was no subsampling in measuring the entire subplot, its σ_s can be assumed to be equal to zero ($\sigma_s = 0$). The 1-m technique σ_s was only 1.39 compared to 3.66 and 3.11 for the 0.3-m and 4-P techniques, respectively.

The subplot measurement on the first three sampling dates gave a dry matter mean of 6.72 Mg ha^{-1}, and the 1-m, 0.3-m, and 4-P methods had dry matter means of 7.05, 9.61, and 9.51, respectively (Table 2). Bias measurements (sign test, t-test, and Eq. [2]) between the 0.3-m and 4-P sample techniques were small, as were measurements of bias between the 1-m and entire subplot-measured techniques (Table 3). Dry matter estimates from either the 0.3-m or 4-P sample techniques were significantly biased from either the 1-m or subplot technique. This is consistent with the suspected overestimation reported by Hanway and Weber (7) for samples of 0.6- and 0.3-m row length.

The 1985 shoot dry matter estimate of 8.85 Mg ha^{-1} was higher than the 1984 value, but the CV for the subplot technique, 0.085, was very similar (Tables 2 and 4). In 1985 the CV's and REMS values for the 0.3-m and 4-P sampling techniques were lower than in 1984, but they were higher for the 1-m technique. The 2-m subplot technique was the least variable with REMS, CV, and σ_s of 1.246, 0.132, and 1.302, respectively.

When measured by the sign test and t-test in 1985, the 0.3-m and 4-P subplot sampling techniques were highly biased for overestimates, but the 1- and 2-m techniques were not biased from the subplot or each other (Table 5). Although not given in Table 5, the estimate of bias and standard error of bias gave similar results to the sign test and t-test. Differences between the subplot measure and the sample measures were not greatly affected by samplers or technicians in 1985 (Table 6).

A significant overestimation bias was present in the 0.3-m and 4-P subplot samples in both years and with different individual samplers or technicians even though our procedure incorporated random selection of samples. The plant population was counted after removal of the shoots for each plot, so overestimation of the population was very unlikely. We can only as-

Table 3. Measures of bias among various sampling techniques in 1984.

	Sampling techniques comparisons†					
Statistic‡	4P-SP	0.3-m-SP	1-m-SP	4P-1-m	0.3-m-1m	4P-0.3-m
	2.787	2.984	0.327	2.461	2.567	0.106
	0.5183	0.6104	0.2319	0.5595	0.5939	0.7198
	0.0001	0.0001	0.1678	0.0001	0.0001	0.8834
	0.0001	0.0016	0.4006	0.0004	0.0300	0.8650

† SP = subplot, 4P = four plants, 0.3-m = 0.3-m row, 1-m = 1-m row.
‡ Estimate of bias = square root of $(\sigma_s^2)n^{-1}$, where n = number of experimental units; P-values = probability of a greater F value under the null hypothesis.

Table 4. Means and estimates of error by five methods of soybean sampling in 1985.

Method	Mean	REMS	CV	σ_s‡
Subplot	8.853	0.755	0.085	0
2-m row	9.462	1.246	0.132	1.302
1-m row	9.353	2.471	0.264	2.308
0.3-m row	12.469	3.969	0.318	3.610
Four-plant	11.389	2.891	0.254	3.343

Table 5. Measures of bias among various sampling techniques in 1985.

	Sampling techniques comparisons†				
Statistic‡	4P-SP	0.3-m-SP	1-m-SP	2-m-SP	1-m-2-m
	2.536	3.617	0.499	0.068	0.109
	0.788	0.851	0.544	0.307	0.368
	0.0050	0.0005	0.3719	0.0641	0.7707
	0.076	0.0014	0.8146	0.0964	1.0000

† SP = subplot, 4P = four plants, 0.3-m = 0.3-m row, 1-m = 1-m row.
‡ Estimate of bias = the mean of the paired differences; SE of bias = square root of $(\sigma_s^2)n^{-1}$, where n = number of experimental units; P-values = probability of a greater test value under the null hypothesis.

Table 6. Difference in the subplot sampling means and subplot means with different technicians conducting the sampling procedure in 1985.

	Sampling techniques comparisons†				
Technician	2-m-SP	1-m-SP	0.3-m-SP	4P-SP	1-m-2-m
A	−0.359	0.424		3.880	0.065
B	1.572	1.524		3.453	0.048
C	0.611	0.397		0.274	0.214
LSD (0.05)	1.336	NS		NS	NS

† SP = subplot, 4P = four plants, 0.3-m = 0.3-m row, 1-m = 1-m row.

sume that as investigators we subconsciously selected for a visualized plant and made consistent selections for bigger plants. Thus, the 0.3-m and 4-P techniques appear to be poor for both precision and bias. The 1-m and 2-m sampling techniques may have eliminated a bias toward more uniform stands that may have caused the overestimation by the 0.3-m technique. Since the 1-m sampling technique was not significantly biased from the subplot sampling technique by the sign test or *t* test, and since it had a 2-yr average CV of less than 0.24, it appears to be an acceptable sampling technique for estimation of soybean shoot growth. The 2-m technique had improved precision, but it had no improvement in the elimination of bias compared to the 1-m technique.

REFERENCES

1. Alberts, E.E., R.C. Wendt, and R.E. Burwell. 1985. Corn and soybean cropping effects on soil losses and C factors. Soil Sci. Soc. Am. J. 49:721-728.
2. Bezdicek, D.F., R.F. Mulford, and B.H. Magee. 1974. Influence of organic nitrogen on soil nitrogen, nodulation, nitrogen fixation, and yield of soybeans. Soil Sci. Soc. Am. Proc. 38:268-273.
3. Bhangoo, M.S., and D.J. Albritton. 1976. Nodulating and non-nodulating Lee soybean isolines response to applied nitrogen. Agron. J. 68:642-645.
4. Burton, J.W., R.F. Wilson, and C.A. Brim. 1979. Dry matter and nitrogen accumulation in male-sterile and male-fertile soybeans. Agron. J. 71:548-552.
5. Carter, T.E., Jr., J.W. Burton, J.J. Cappy, D.W. Israel, and H.R. Boerma. 1983. Coefficients of variation, error variances, and resource allocations in soybean growth analysis experiments. Agron. J. 75:691-696.
6. ———, D.C. Cooper, and J.W. Burton. 1984. Optimum plot size in soybean growth analysis experiments. Agron. Abstr. American Society of Agronomy, Madison, WI, p. 61.
7. Hanway, J.J., and C.R. Weber. 1971. Dry matter accumulation in eight soybean [*Glycine max* (L.) Merrill] varieties. Agron. J. 63:227-230.
8. Hunt, P.G., T.A. Matheny, and A.G. Wollum II. 1985. *Rhizobium japonicum* nodular occupancy, nitrogen accumulation, and yield for determinate soybean under conservation and conventional tillage. Agron. J. 77:579-584.
9. Johnson, J.W., L.F. Welch, and L.T. Kurtz. 1975. Environmental implications of N fixation by soybeans. J. Environ. Qual. 4:303-306.
10. Karlen, D.L., P.G. Hunt, and T.A. Matheny. 1982. Accumulation and distribution of K, Ca, and Mg by selected determinate soybean cultivars grown with and without irrigation. Agron. J. 74:347-354.
11. Kohl, D.H., G. Shearer, and J.E. Harper. 1980. Estimates of N_2 fixation based on differences in the natural abundance of ^{15}N in nodulating and non-nodulating isolines of soybeans. Plant Physiol. 66:61-65.
12. Matheny, T.A., and P.G. Hunt. 1983. Effects of irrigation on accumulation of soil and symbiotically fixed N by soybean grown on a Norfolk loamy sand. Agron. J. 75:719-722.
13. Nelson, A.N., and R.W. Weaver. 1980. Seasonal nitrogen accumulation and fixation by soybeans grown at different densities. Agron. J. 72:613-616.
14. Patterson, T.G., and T.A. LaRue. 1983. Nitrogen fixation by soybean: Seasonal and cultivar effects and comparisons of estimates. Crop Sci. 23:488-492.
15. Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Company, Inc., New York.